

Full Length Research Paper

Hypoglycemic profile and ameliorative potential of aqueous garlic extract on sperm characteristics in glibenclamide treated diabetic male rats

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This study was carried out to determine the protective effect of aqueous garlic extract on testicular and spermatogenic changes in glibenclamide treated diabetic male rats. Thirty matured male rats were used in this study and were assigned into five groups of six rats each. Diabetes was induced in groups 1, 2, 3, 5 but not induced in group 4. Rats in group 1 were treated with glibenclamide (0.6 mg/kg) daily for 21 days. Rats in group 2 were treated with glibenclamide (0.6 mg/kg) and garlic extracts (*Allium sativum*) at the dose of 300 mg/kg for 21 days. Rats in group 3 were untreated diabetic given distilled water. Rats in group 4 were the normal control, given distilled water. Rats in group 5 were treated with garlic (300 mg/kg) dissolved in distilled water. On day 21 post treatment, there was a significant ($p < 0.05$) decrease in the fasting blood sugar (FBS) level of glibenclamide treated group when compared to garlic treated group and diabetic untreated group but there was a significant ($p < 0.05$) decrease in the FBS level of co-administration of glibenclamide and garlic when compared to glibenclamide alone and garlic alone. There were significant ($p < 0.05$) increases in testicular sperm count, epididymal sperm count and percentage sperm motility of group 2 when compared to groups 1, 3 and 5. From the above result, co-administration of glibenclamide and garlic extract produced optimum hypoglycemic activity and protective effect on testicular sperm and epididymal sperm counts, and percentage sperm motility in diabetic male rats.

Key words: Diabetes, fasting blood sugar (FBS), garlic, glibenclamide, sperm count, sperm motility.

INTRODUCTION

Diabetes is a metabolic disease characterized by high blood sugar (FBS) levels, either, because the body does

not produce enough insulin or because the body cells do not properly respond to insulin that is produced (Rother,

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2007). Insulin is a hormone produced by the beta cells of the pancreas. Its primary function is to transport glucose to cells. If this function cannot be met as in diabetic cases glucose accumulates in the blood leading to many complications (Rother, 2007). Diabetes mellitus has classical signs of frequent urination (polyuria), increased thirst (polydipsia), increased hunger (polyphagia) and weight loss. There are two main types of diabetes, which includes Type 1 and Type 2 diabetes mellitus. Type 1 diabetes mellitus which results from the body's failure to produce enough insulin due to loss of insulin producing beta cells of the pancreas. This form is also referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile onset diabetes". Majority of this Type 1 is of immune mediated nature where the beta cells loss is due to a T-cell mediated autoimmune attack (Thomas and Philipson, 2015).

Type 2 diabetes mellitus, begins with insulin resistance. A condition in which cells fail to respond to insulin properly; as the disease progresses a lack of insulin may also develop (James and Luke, 2009). This form is also referred to as "non-insulin dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes".

Diabetes mellitus is a disease of both humans and animals. In animals, diabetes mellitus is more common in dogs and cats (Baker et al., 1983) and laboratory rodents (Thomas et al., 1997). The disease has also been reported in horses, cattle, pigs, sheep and guinea pigs (Thomas et al., 1997).

Male reproductive organ alterations have been widely reported in both man and animals with diabetes. About 90% of diabetic males have changes in testicular and spermatogenic parameters, including decreases in testicular weight, percentage sperm motility, testicular and epididymal sperm reserves due to testicular dysfunction associated with sustained hyperglycemia (AbuAbeleh et al., 1984; Orth et al., 1979; Paz and Homonnai, 1979; Hurtado de Catalfo et al., 1998). Alloxan induced diabetic male rats exhibit decreases in testicular parameters after 2 weeks of induction of diabetes (Sanguinetti et al., 1995). Zhao et al. (2011) demonstrated that oxidative stress induced by hyperglycemia is the major cause of diabetic testicular damage as oxidative stress is increased in diabetes, due to the overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defences (Ballester et al., 2004). The statement of the problem include: (1) There is no synthetic agent that perfectly improves FBS levels and spermatogenic alterations associated with diabetes in males thus, the need to evaluate concurrent use of synthetic and herbal remedies in the management of diabetes for possible physiological benefits. (2) Growing evidence has shown that diabetes mellitus has negative effect on male reproduction hence the search for a protective agent has become an area of

active research. Therefore, the aim of this study was to investigate the hypoglycemic activity and protective effect of aqueous garlic extract on sperm characteristics in glibenclamide treated diabetic male rats.

MATERIALS AND METHODS

Animals

Thirty mature male albino Wistar rats (*Rattus norvegicus*) of 12 weeks old weighing between 180 to 200 g were used for the study. They were sourced from Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in clean cages at room temperature (37°C) and were fed *ad libitum* on a standard commercial grower feed (Vital feeds, GCOM Nig. Ltd) and clean drinking water. The animals were maintained under a cycle of 12 h of light and 12 h of darkness daily throughout the period of experiment. The ethical rules governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997) and Zimmermann (1983).

Ethical approval

Guidelines for the care and use of experimental animals complied with the University animal welfare guidelines and policies and were approved by the ethical committee of University of Nigeria, Nsukka (approval ref no. 20170704)

Experimental design

Thirty mature male albino Wistar rats (*R. norvegicus*) of 12 weeks old weighing between 180 to 200 g were used for the study. They were fasted overnight and their blood glucose levels (normoglycaemic levels) were determined using Accu-check glucometer (Roche, Germany). Diabetes was then induced in groups 1, 2, 3 and 5, but not in group 4 and treated as follows daily for 21 days: Group 1: rats in this group were diabetic male rats treated with glibenclamide (0.6 mg/kg) dissolved in distilled water; group 2: rats in this group were diabetic male rats treated with glibenclamide (0.6 mg/kg) and garlic extract (300 mg/kg) dissolved in distilled water; group 3: rats in this group were diabetic untreated rats given distilled water; group 4: rats in this group were the normal control; group 5: rats in this group were diabetic male rats treated with only garlic extract (300 mg/kg) dissolved in distilled water.

Preparation and extraction of plant materials

The plant was acquired from Orba market. Fresh garlic (*Allium sativum*) was dried. The fresh garlic was crushed using mortar and pestle into pasty materials. Cold extraction of the pasty garlic material was performed using distilled water. The extract was filtered using Wattman no 1 filter paper. The filtrate was stored in the refrigerator before it was finally used.

Induction of diabetes

The basal fasting blood sugar of each animal was established using Accu-check active glucose test strip. Diabetes was induced in rats

Table 1. Hypoglycemic Study.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
FBS day 0 (mg/dl)	160.33±10.45 ^a	165.66±5.55 ^a	164.66±6.96 ^a	87.00±7.02 ^a	156.67±7.69 ^a
FBS day 7 (mg/dl)	89.33 ±5.21 ^{ab}	79.09±0.58 ^b	169.37 ±6.36 ^c	86.67±6.77 ^{ab}	125.33 ±2.60 ^d
FBS day 14 (mg/dl)	91.00±3.79 ^a	73.33±1.45 ^b	169.34±6.36 ^c	86.67±6.56 ^{ab}	119.67±1.45 ^d
FBS day 21 (mg/dl)	92.33±3.84 ^a	72.33±1.45 ^b	186.67±6.96 ^c	87.33±6.01 ^a	118.67±1.33 ^d

^{a,b,c,d}Different superscripts along the same column express significant ($p < 0.05$). FBS, Fasting blood sugar.

using the method described by Venugopal et al. (1998). Diabetes was induced in overnight fasted male rats by a single intraperitoneal injection of freshly prepared solution of alloxan-monohydrate (160 mg/kg body weight). The fasting blood sugar levels of the rats were determined daily until diabetes was confirmed. Rats with blood sugar levels above 126 mg/dl were considered diabetic (Iwalewa et al., 2008).

Determination of testicular weights

At the end of the 21 days of treatment (end of study period), the rats in each treatment group as well as the control were euthanized using euthetal (180 mg/kg) intraperitoneally. The testis from each rat was carefully dissected out, trimmed free of extraneous tissues and weighed with a weighing balance.

Determination of gross percentage sperm motility

This was done using the method described by Hotchkiss et al. (1952). A drop of sperm sample from the epididymis was placed on a clean slide, covered with a cover slip and viewed under the microscope at X40 magnification (Olympus xxx). Then, motile sperm cells were determined per 100 sperms seen. Two counters were used: one for a total of 100 sperms, the second for motile sperms.

Determination of epididymal sperm reserve

This was done using the method described by Amann (1986). The left and right caudal epididymis were crushed with ceramic mortar and pestle, 10 ml of normal saline added to each and filtered through a nylon sieve. Each filtrate (0.1 ml) was further diluted with 0.9 ml of white blood cells diluting fluid in a test tube and 20 μ l of each diluted sperm solution was used to charge the improved Neubauer chamber (Germany) and viewed under the microscope at x40 (Olympus xxx). The number of sperm cells were counted on the four corner squares and estimated in 169 squares. As these sperm cells were counted in 2.5×10^{-4} ml, which is the volume of the Neubauer chamber, the number of sperm cells counted in each sample was multiplied by 10 to get the total number of sperm cells in 10 mls of normal saline.

Determination of the testicular sperm reserve

This was done with the two testicles using the method of Amann, 1986 already described.

RESULTS

Hypoglycemic study

The anti-hyperglycemic study reveals that on day 0, there was no significant difference in fasting blood glucose level among the diabetes induced groups. On day 21 post treatment, there was a significant decrease in the FBS level of glibenclamide treated group (group 1) when compared to garlic treated group (group 5) and diabetic control (group 3) but there was a significant decrease in the FBS level of co-administration of glibenclamide and garlic (group 2) when compared to glibenclamide alone (92.33±3.84) and garlic alone (Table 1).

Determination of testicular weight, epididymal and testicular sperm reserves and percentage sperm motility

There were significant ($p < 0.05$) increases in testicular sperm count, epididymal sperm count (Table 2) and percentage sperm motility (Figure 1) of group 2 when compared to group 1.

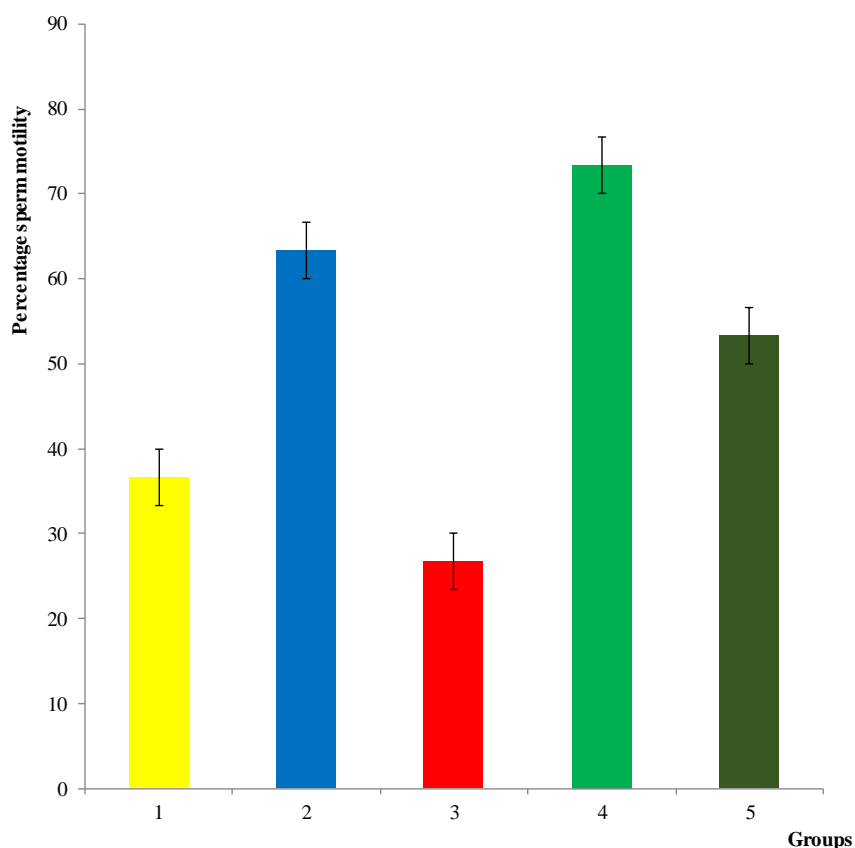
DISCUSSION

The result of anti-hyperglycemic study revealed that on day 0, there was no significant difference in fasting blood glucose level among the diabetes induced groups. This showed that diabetes was confirmed on day 0 in induced groups. On day 21 post treatment, there was a significant ($p < 0.05$) decrease in the FBS level of glibenclamide treated group when compared to garlic treated group and diabetic untreated group (186.67±6.96) but there was a significant ($p < 0.05$) decrease in the FBS level of co-administration of glibenclamide and garlic when compared to glibenclamide alone and garlic alone. This significant higher glycemic control of co-administration of glibenclamide and garlic may be due to synergistic drug-dietary interactions. The observed decreases in testicular weight, testicular sperm reserve,

Table 2. Testicular weight and sperm counts.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
TW(g)	1.20±0.06 ^a	1.37±0.23 ^a	0.83±0.03 ^b	1.57±0.12 ^a	1.17±0.03 ^c
TSR(10 ⁶)	11.33±0.35 ^a	17.43±0.64 ^b	8.3±0.26 ^c	20.23±0.46 ^d	11.0±0.40 ^a
ESR(10 ⁶)	13.90±0.06 ^a	19.97±0.12 ^b	8.7±0.35 ^c	22.40±0.38 ^d	13.63±0.32 ^a

^{a,b,c,d}-Different superscripts along the same column express significant ($p < 0.05$). TW, testicular weight; TSR, testicular sperm reserve; ESR, epididymal sperm reserve.

**Figure 1.** Mean percentage sperm motility.

epididymal sperm reserve and percentage sperm motility in the untreated diabetic male rats in this study agreed with those of earlier reports (Abuabeeleh et al., 1984; Hurtado de Catlfo et al., 1998; Anderson and Thliveris, 1986; Rossi and Aeschlimann, 1982; Orth et al., 1979; Paz and Homonnai, 1979). The significant ($p < 0.05$) increases in testicular sperm count, epididymal sperm count and percentage sperm motility of group 2 when compared to group 1- testicular sperm count, epididymal sperm count and percentage sperm motility may be due to the antioxidant activity of aqueous garlic extract (Borek

et al., 2001).

Oxidative stress and lipid peroxidation is known to play a major role in the etiology of the defective reduction of sperm count and decline in cell quality which results in insufficient numbers of viable spermatozoa and infertility in diabetic males (Boonsorn et al., 2010).

Garlic contains antioxidant, selenium which scavenges reactive oxygen species and inhibits lipid peroxides in the body (Borek et al., 2001; Chang et al., 1980). In this study, garlic improved sperm count and cell quality in glibenclamide treated diabetic male rats.

Conclusion

In conclusion, co-administration of glibenclamide and aqueous garlic extract produced optimum hypoglycemic activity and protective potential on spermatogenic changes in alloxan induced diabetic male rats. Since garlic is available in our environment, its consumption in addition to glibenclamide (antihyperglycemic agent) will be an effective way to reduce the toxic effects of diabetes on the reproductive system of males, and by so doing, will help in improving fertility in diabetic males.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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